Extracellular Microbial Polysaccharides

New hydrocolloids of interest to the food industry

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□ THE POLYSACCHARIDE HYDROCOLLOIDS (designated polysacolloids by Stoloff, 1958), used traditionally for many purposes in foods and food processes, have been obtained from plants, both land and aquatic. An additional newly developing source of polysacolloids of demonstrated or apparent suitability for use in foods is based on the biosynthetic capabilities of nonpathogenic microorganisms. Research at the Northern Regional Research Laboratory has augmented awareness of the great numbers of microbial species, nonpathogenic to man, that produce polysaccharides both extracellularly and abundantly. Our research also has extended cognizance of the diversity of the polysaccharide composition and structure, and has established that the properties may be not only unusual but also amenable to practical utility.

Interest in this new source of hydrocolloids stems from several considerations which are: 1) Successful performance of several of these microbial products based on their distinctive properties, 2) general suitability for ingestion without adverse effects because the constituent natural sugar residues and their pattern of glycosidic linkages permit either digestion and metabolism or inertness and noncaloric effect, 3) economic feasibility resulting from the extracellular occurrence of these biopolymers and their production in good yields from low-cost substrates by fermentation procedures, 4) the need to supplement supplies of the natural plant gums, and 5) the various advantages of domestic products over foreign imports.

Several comprehensive reviews have detailed the microbiology, production, characterization and applications (both actual and proposed) of extracellular microbial polysaccharides (Jeanes, 1966, 1968, 1973) and the prognosis for their potential in the food industry (Glicksman, 1969). Reviewed here are some of the basic research and the associated industrial developments on extracellular microbial polysaccharides as related to the interests of the food industry.

The extracellular microbial polysaccharides that have been most studied are members of several classes representative of the neutral and polyanionic types. Research on the polycationic type is just beginning (Jeanes, et al., 1971). The polysaccharides now in industrial production are dextran, which is a neutral glucan, and xanthan, a polyanionic heteropolysaccharide. These biopolymers and a few others of their respective types are the subjects for further consideration here.

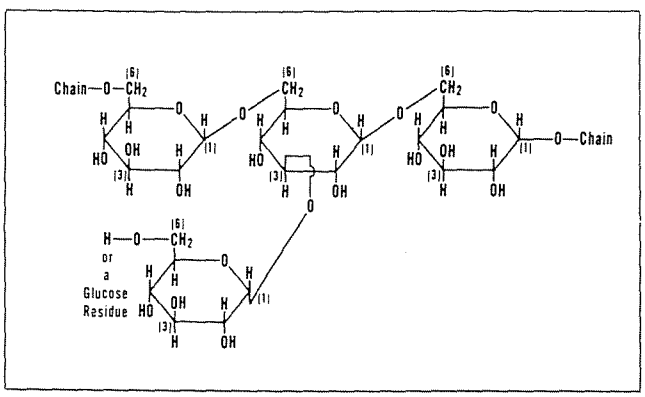

Fig. 1—**DEXTRAN** *from* Leuconostoc mesenteroides *NRRL B-512(F) has a flexible uniformly linked backbone structure and infrequent branches predominantly one or two glucose residues long.*

DEXTRAN

The dextran now produced in the United States, Canada, Sweden, and elsewhere is synthesized from sucrose by the extracellular enzyme, dextransucrase, from *Leuconostoc mesenteroides* strain NRRL B-512(F). (Unless stated otherwise in this discussion, the term "dextran" designates the product from this specific strain. Dextrans from other strains differ in structure and properties.) Cell-free culture filtrates containing the enzyme may be used; the resultant solution contains only the dextran product, the enzyme and residual nutrients from the culture filtrate, and the by-product, fructose. Partial depolymerization of this "native" dextran, which has molecular weight in the approximate range of 30–90 million, and subsequent fractionation, permits production of a series of lower molecular weight products which are marketed for pharmaceutical and industrial uses (Jeanes, 1966).

This dextran is composed exclusively of α-D-glucopyranosidic residues, 95% of which are linked only through carbons 1 or 1 and 6; 5% carry branches predominantly one or two glucose units long attached at carbon 3 positions (Fig. 1).

Tests on metabolism of NRRL B-512(F) dextran have been made mainly on the clinical-size fraction of $\overline{M}_w$ 75,000 ± 25,000. When infused intravenously to man or animals, metabolism is complete (Terry et al., 1953); when ingested, it serves as a source of liver gly-

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Table 1—**VISCOSITY VS. CONCENTRATION** *relations of native dextran from* Leuconostoc mesenteroides *NRRL B-512(F) at 25°C, 3.84 sec^{-1}.*

Dextran concentration (%)	Viscosity (cp)
1	13
2	80
3	220
4	300
5	400

cogen (Bloom and Wilhelmi, 1952). Comparison of products of $\overline{M}_w$ 75,000 and 10 × 10^6 in feeding tests with rats showed digestibility to be 90% and 86%, respectively, and other observations were essentially alike (Booth et al., 1963). There is no scientific basis for expecting significant difference in digestibility of partially degraded and native dextrans. Breakdown in the intestinal tract is determined by whether dextranase is present and the structure (degree of branching) of the dextran, and not by molecular size. Dextranases occur in intestinal mucosa of man and other mammals (Dahlqvist, 1961, 1963), in the colon as a product of certain of the normal flora (Sery and Hehre, 1956) and in various tissues, especially spleen, liver and kidney (Ammon, 1963).

For practical use in foods, costs favor native dextran. Action by Food and Drug authorities in the United States (FDA), however, has been based on the tests with fractions of partially degraded dextran and the lack of adequately supported petitions for approval of native dextran. Dextrans of average molecular weight below 100,000 were included among GRAS products (FDA, 1960) until their proposed deletion because of disuse (FDA, 1973b). In Canada, also, approved use has been restricted to molecular weight below 100,000.

Dextran NRRL B-512(F) dissolves readily in water to give clear solutions which are stable to sterilization by heat and to freezing and thawing. Viscosity-concentration relations are shown in Table 1 and compared with those of polyanionic polysaccharides of similar molecular weight in Figure 2. In order to compare the flow behavior of dextran with that of 1% solutions of other polysacolloids under our test conditions (Jeanes and Pittsley, 1973, 15% concentration was necessary (Fig. 3). In terms defined for this system (Patton, 1969) the dextran solution shows plastic characteristics closely similar to those of 1% locust bean gum. The same similarity was observed by Szczesniak and Farkas (1962) and related to mouthfeel characteristics. The influence of concentration on these fundamental flow characteristics has been considered by both Szczesniak and Farkas (1962) and Patton (1969). We found that dextran solution at 5% concentration showed essentially Newtonian-type flow.

A number of other well-defined characteristics of this dextran are bases for uses in the food industry as detailed in specific examples by Jeanes (1967) and Glicksman (1969). The notorious role of dextrans as nuisances in the cane sugar industry attest to general ability to interfere with crystallization of sugar solutions. Aqueous solutions of the B-512(F) dextran dry to water-soluble, cohesive films. "Crystalline" regions that would diminish solubility do not develop during aging of the native material in the presence of mois-

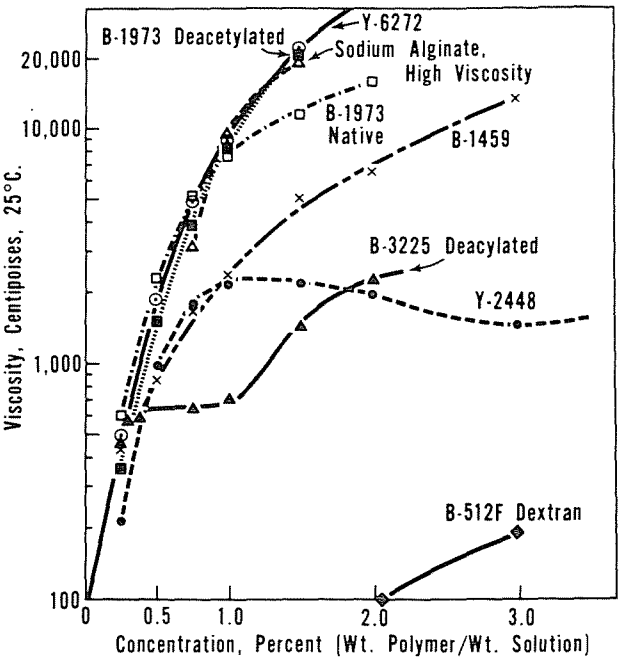

Fig. 2—**VISCOSITY CONCENTRATION CURVES** *for microbial polysaccharides and an alginate. (Wells-Brookfield Micro Viscometer [Cone and Plate, Model RVT], 3.84 sec^{-1} (1 rpm), 25°C.)*

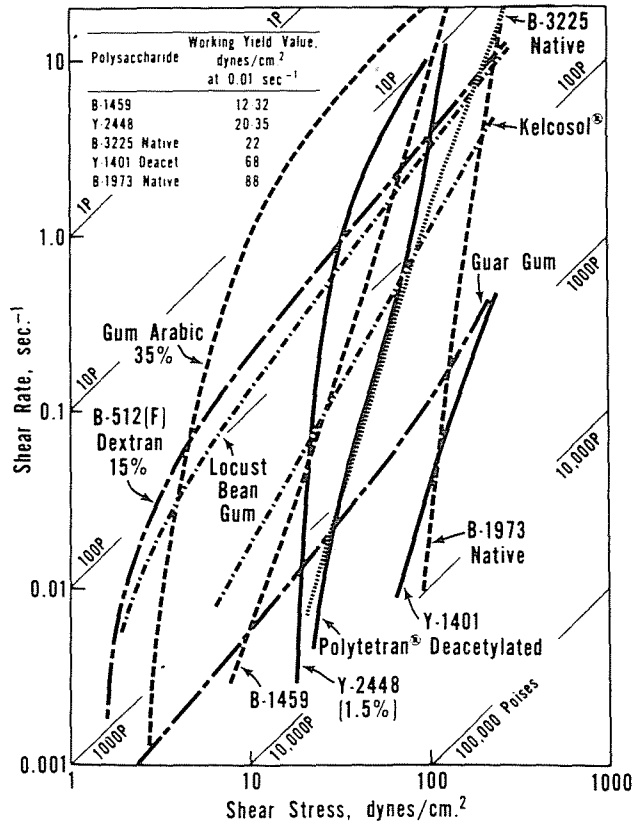

Fig. 3—**SHEAR STRESS-SHEAR RATE-VISCOSITY** *relations for aqueous dispersions of microbial polysaccharides and plant gum controls. Concentrations were 1% except as indicated. (Wells-Brookfield Micro Viscometer [Cone and Plate, Model RVT], 25°C.)*

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Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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(FDA, 1971), and as an optional stabilizing ingredient in certain cheeses and cheese products (FDA, 1973a). General use of xanthan gum in foods has been approved also in Canada and in several other countries, and petitions are pending in still more countries (Kelco, 1972).

Fundamental characteristics of xanthan provide the bases for its numerous applications (Kelco, 1972) in food products and food processing. Its homogeneous aqueous dispersions have high viscosities at low concentrations, while at higher concentrations its property of molecular self-interaction and weak gelation is manifested (Fig. 2). In dilute solution its viscosity is much less affected by salts, low pH and heat than that of plant polyelectrolytes. Its dispersions thin readily with shear and are nonthixotropic, yet have high yield value (Fig. 3). Thus, xanthan dispersions have excellent properties of flow (Stanislav and Sheets, 1971; Rocks, 1971), mouthfeel and flavor release (Szczesniak and Farkas, 1962), and suspending ability (Kelco, 1972). Xanthan is compatible with most other hydrocolloids; it enhances the behavior of starch and, with locust bean gum, shows a unique viscosity-increasing and gelation effect (Rocks, 1971; Kovacs, 1973) which is under fundamental investigation (Rees, 1972). Its formation of cohesive, flexible films (Jeanes, 1967, 1973) would contribute favorably to its role in emulsion and foam stabilization (Araujo, 1967; Kelco, 1972), and to possible usefulness for coatings and encapsulation. The American Institute of Baking (personal communication, 1973) has shown that, when used in gluten-free bread, a low concentration suffices to achieve desirable loaf volume, crumb texture and crust quality. In this use in bread, as well as in French-type dressings (Anon., 1971) and most other food products, the concentration of xanthan is lower and its performance is superior to those of substances used previously.

OTHER MICROBIAL POLYANIONIC POLYSACCHARIDES

Many water-soluble microbial polyanionic polysaccharides are known; the few that have been investigated sufficiently to indicate possible potential for food applications are listed in Table 2. Polysaccharide B-1459 (xanthan) is included for comparison; FDA clearance has not been sought for any of the others. The compositions vary considerably and are especially noteworthy for polysaccharides from strains B-1973, B-3225, Y-2448 and Y-6272, which represent types previously unknown. Treatment with dilute alkali removes *O*-acetyl and succinyl ester substituents; obtained in this way, deacylated polysaccharide B-3225 is unique in having pyruvic acid ketal as its only acidic group.

Individuality shown in the viscosity vs. concentration relationships (Fig. 2) is indicative of fundamental differences that would favor specific uses. The flow curves (Fig. 3) are also distinctive for each substance. These curves were obtained by Jeanes and Pittsley (1973), who used the technique of Patton (1969) as well as his criteria for classification. All of the polyanionic polysaccharides from NRRL strains are shear thinning, xanthan (B-1459) and phosphomannan Y-2448 are outstandingly so. All are nonthixotropic and nondegradative under shear that might normally be applied. Curves are not shown for four pseudoplastic substances: B-1973 deacetylated and Y-6272, which parallel at somewhat lower viscosities the respective curves for guar and locust bean gums, and the deacylated forms of B-1797 and B-3225. Xanthan shows some aspects of both plastic and pseudoplastic substances. All the others included in Figure 3 (Y-2448, Y-1401 deacetylated, and native forms of B-1973 and B-3225) are plastic, as are two not included (native forms of Y-1401 and B-1797). These plastic substances, after their respective yield value is exceeded, flow more readily than do the pseudoplastic controls (Kelcosol®, and locust bean and guar gums) and the plastic gum arabic. This array of hydrophilic polymers displaying plastic properties at relatively low concentrations is unusual since few have been found previously, either among the natural or synthetic substances. Shear stress at 0.01 sec^{-1} provides a measure of yield value (Patton, 1969); the microbial polysacolloids having high values exhibit good suspending ability.

Among these microbial polyelectrolytes, only phosphomannan Y-2448 shows typical decrease in solution viscosity in the presence of salts; the others show restrained decrease, or increase, depending on the polysaccharide and salt concentrations. Phosphomannan Y-2448 forms brilliantly clear solutions which, although biodegradable, are exceptionally resistant to spontaneous microbial contamination. In low concentrations, it has a "profoundly beneficial effect" as a stabilizer of foam in beer and other carbonated alcoholic malt beverages (Segel, 1960). Phosphomannan Y-2448 has a high mouthfeel rating (Szczesniak and Farkas, 1962) and, when fed to rats, was partially utilized without producing pathology, but showed cathartic effects typical of hydrophilic polysaccharides (Booth et al., 1963). Polysaccharide B-3225 is outstanding for the stability of its solution viscosity to heat, and polysaccharide B-1973, both native and deacetylated, shows exceptional flexibility of films cast from aqueous solutions.

Thus, pioneering research on extracellular microbial polysaccharides has disclosed new economical sources of hydrophilic colloids which are diverse in composition and have a spectrum of unusual physical properties. The rapid growth in use and the highly beneficial performance of xanthan gum in many phases of food production, should encourage the exploration of uses of other microbial polysaccharides by the industry.

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ture (Taylor et al., 1959) although tendency to "retrogradation" increases as molecular weight decreases.

Much fundamental research has been done on the interaction of B-512(F) dextran, especially fractions such as the clinical-size, with proteins of blood serum and with mucopolysaccharides (Laurent, 1966). The mechanism of these interactions, unlike that of dextran antigen-dextran antibody reactions, does not involve specific complemetary binding sites, but pervasion of solution space by dextran molecules excludes other macromolecules and thus diminishes their activity and solubility (Laurent, 1966). The mechanism of the complex interaction between dextran B-512(F) and wheat gluten has not been resolved, but the properties of the gluten are modifid (Wilham et al., 1959; Jones and Erlander, 1967). When dextran is used as a component in bread, the proportion of gluten may be increased and loaf volume, moisture retention, and shelf life are improved (Bohn, 1961).

OTHER NEUTRAL MICROBIAL POLYSACCHARIDES

Two types of β-1,3-glucans may have applicability in food preparations. Both are insoluble in water but hydrate and swell greatly; they have exceptional stability to heat and acid but are labile to alkali. One of these is a β-glucosyl glucan Polytetran® (Pillsbury Co., Minneapolis, Minn.) which has branches one unit long attached by 1,6-bonds to the 1,3-linked main chain (Pillsbury, 1970). The other (curdlan) is unbranched; when heated in water it forms a firm resilient gel which, unlike agar gels, maintains its structure when either hot or cold (Harada et al., 1966).

XANTHAN

The polyanionic heteropolysaccharide popularly called xanthan gum is produced usually from dextrose in growing cultures of *Xanthomonas campestris* NRRL B-1459. A process for continuous fermentation has been established by Rogovin (1969). For a food-grade product, purification involves clarification of the fermentation broth by centrifugation, precipitation of the xanthan usually by a water miscible alcohol to separate it from solubles of the culture medium, reprecipitation if desired, and dehydration by drum or spray drying, use of an alcohol or lyophilization (Sohns et al., 1966).

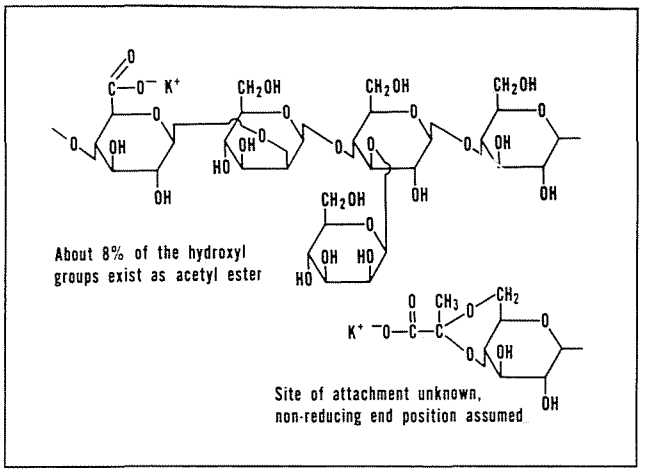

Fig. 4—**XANTHAN GUM STRUCTURAL FEATURES** *showing the known structures, linkages, and sequences of sugar units. The repeat unit is not precisely established.*

We have prepared this polysaccharide as the neutral potassium salt, but other cations might be used. As an indication of purity obtainable, a pilot-plant product dehydrated by methanol has, in percent: N, 0.4; P, 0.1; and ash, 12.0. The theoretical ash value is about 11%.

The composition of xanthan is included in Table 2 and its main structural features are shown in Figure 4. The molecular weight of our preparations has been around 5 to 10 million.

Feeding tests on rats (Booth et al., 1963) and dogs (Robbins et al., 1964) showed that xanthan is not utilized, it has no growth-inhibiting factor and caused no acute toxicity, skin irritation or sensitization. Dogs showed catharsis and a dramatic lowering of cholesterol, especially when fed at the highest test level. Further extensive toxicological and other testing has established the safety of xanthan for food use (Kelco, 1972).

Action by the United States Food and Drug Administration has cleared xanthan gum as a food additive where such use is not precluded by Standards of Identity Regulations (FDA, 1969), as an optional emulsifying and stabilizing ingredient in French dressing

Table 2—**COMPONENTS** *of microbial extracellular anionic polysaccharides and their molar proportions*[a]

NRRL strain No.	Identity of microbial source	Anionic component[c] (moles)				Neutral sugar component (moles)				*O*-Acetyl (moles)
		Gluc UA[b]	Man UA	Pyruvic acid[d]	Other[d]	Gluc	Gal	Man	Other	
B-1459	*Xanthomonas campestris*	2	—	0.6	—	3	—	3	—	1.7
B-1797	*Arthrobacter viscosus*	1	—	1	—	3	3	—	—	3
B-1973	*Arthrobacter viscosus*	—	1	—	—	1	1	—	—	5
B-3225	*Arthrobacter stabilis*	—	—	0.3	Succinic acid, 0.3	2	1	—	—	0.5
Y-1401	*Crystococcus laurentii* var. *flavescens*	1	—	—		—	—	4	Xylose, 1	1.7
Y-2448	*Hansenula holstii*	—	—	—	Orthophosphoric, 1	—	—	5	—	—
Y-6272	Unidentified black yeast	—	—	—	*N*-Acetyl glucosaminuronic, 1	—	—	—	*N*-Acetyl glucosamine, 2	—

[a] Research data from NRRL (Jeanes, 1968, 1973; Jeanes et al., 1971).
[b] Abbreviations: Gluc, glucose; Gal, galactose; Man, mannose; Gluc UA, glucuronic acid; Man UA, mannuronic acid.
[c] Present as the neutral potassium salt.
[d] Combined as pyruvic ketal, succinic half-ester, and phosphoric diester.